

Effect of Arbuscular Mycorrhizal Fungi (AMF) on the Yields of Pepper (*Capsicum annum* L.) under Protected Conditions

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Citation: Toledo Cabrera, B., Montero Limonta, G., & Bazán Delgado, A. (2020). Effect of Arbuscular Mycorrhizal Fungi (AMF) on the Yields of Pepper (*Capsicum annum* L.) under Protected Conditions. *Agrisost*, 26(1), 1-11. Recuperado a partir de <https://revistas.reduc.edu.cu/index.php/agrisost/article/view/e3353>

Received: April 1, 2019

Accepted: December 22, 2019

Published: January 28, 2020

Funding source: undeclared.

Conflicts of interest: no conflict of interest has been declared.

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Abstract

Context: Pepper yields have declined in recent years due to the impact of several factors, including the presence and little availability of nutrients in the soil, and the lack of efficient strains of AMF in the technological package. The application of AMF strains in the nutritional process of pepper improves nutrient availability in the soil, and increases crop yields.

Aim: To evaluate the yields of pepper (*Capsicum annum* L.) inoculated with efficient strains of arbuscular mycorrhizal fungi (AMF)

Methods: Direct inoculation of microorganisms during transplantation with a 10% proportion, depending on the root-ball volume. A completely randomized experimental design was used, with four treatments, and four repetitions, with a control that was not inoculated, and three levels of inoculation with *Rhizophagus intraradices* (INCAN-11), *Glomus cubense* (INCAN-4), and *Funneliformis mosseae* (INCAN-2). The zigzag sampling of fungal variables was done along the field, and several variables were evaluated: root colonization percent, visual density, and spore contents. Additionally, plant and fruit height and thickness were determined, and the fruits were weighed to determine crop yields.

Results: The best results were achieved with the *Glomus cubense* (INCAN-4) (T3) strain. The results observed showed the efficacy of the third treatment, which produced a cost-effectiveness of \$ 3.13.

Conclusions: The AMF strains used had a favorable behavior in relation to the indicators evaluated: growth, development, and crop yield. The application of *G. cubense* (INCAN-4) produced the best pepper (*Capsicum annum* L.) yields.

Key words: pepper, inoculation, arbuscular mycorrhizal fungi, symbiosis, yields.

Introduction

Pepper is one of the most important crops in Cuban horticulture, but yields have declined in recent years due to several factors, such as water shortage, inappropriate use of chemical fertilizers, and little availability of soil nutrients, as the most significant ones.

In Cuba, pepper is one of the protected horticultural crops covering the largest cultivated areas. The demand of fresh peppers throughout the year has grown remarkably, producing an increase in

protected conditions for this crop. In 2017, pepper production in Cuba was 70 202 tons. According to ONEI 2016, in Santiago de Cuba, pepper yields in the state-owned sector accounted for 156.7 t/ha. Particularly, in Campo Antena protected cultivation field, the average yields of variety Lucomone represented 30.5 t/ha.

Today, in the world, protected cultivation is developed through advanced technologies that contribute to effective production of fresh greens throughout the year. The yields of green vegetables observed in some protected cultivation projects in

Cuba have shown important qualitative and quantitative changes compared to exposed crops (Casanova et al., 2007).

Protected cultivation facilities ensure high yields and stable supplies. The utilization of bioproducts with biorregulating and biostimulating functions in crop growth is the base of soil fertility (Rodríguez et al., 2011).

The inclusion of microorganisms such as biostimulants and biofertilizers for biological control of diseases is a sustainable alternative for integrated crop management. Several microbial species have been isolated and used in agriculture thanks to their antagonistic effects. Jiménez, Ramírez & Mena (2014) isolated strains of *Bacillus subtilis* F16/95, *B. subtilis* Xph, and *Pseudomonas putida* 14A from the rhizosphere of potato (*Solanum tuberosum* L.), maize (*Zea mays* L.), and beans (*Phaseolus vulgaris* L.). In their experiments, they evaluated the antagonistic effects of these strains for biological control of bacterial blight of cucurbitaceae, bacterial blight in water melon fruit, and the rotting of roots, caused by *Xanthomonas cucurbitae*, *Acidovorax avenae* sub sp. *citrulli* and *Fusarium oxysporum*, respectively (Jiménez, Mena & Ramírez, 2014).

One of the alternatives to increase crop production is the application of biofertilizers containing arbuscular mycorrhizal fungi (AMF), which establish a symbiosis with the roots and play important roles, contributing to efficient crop survival and growth, in addition to reducing the effects of stress associated to nutrition, and interaction with water. In that sense, the formation of micorhyzae has an important role in the growth of plants under water stress (Montero, Duarte, Cun & Cabrera, 2010).

They can also increase plant tolerance to several factors of abiotic stress, such as droughts, excessive levels of toxic elements, salinity, unbalances or nutrient deficiencies. Some greens that require nursing in initial stages, such as Chile pepper (*Capsicum annuum* L.), might be benefited with the inoculation of AMF.

Among the nutritional alternatives, the utilization of biofertilizers and arbuscular mycorrhizal fungi have been very efficient as mineral fertilizers; their combination with organic matter produces greater efficacy due to their synergistic effect (Charles & Martín, 2015).

Experiments done by Bell-Mesa, Osoria-Galan, Montero-Limonta & Molina-Lores, (2017), showed favorable results in indicators of germination, plant height, stem thickness, survival counts, colonization, and visual density, when *Glomus cubense* (INCAN-

4) was applied to pepper seedlings (*Capsicum annuum* L.).

Previous chemical analyses performed to the soil at Campo Antena protected cultivation facility, from America Libre Socialist Company, in Santiago de Cuba, found little nutrient availability, causing a decline in pepper productivity.

Accordingly, the aim of this research was to evaluate the effect of different strains of arbuscular mycorrhizal fungi (AMF) on pepper (*Capsicum annuum* L.) yields.

Materials and Methods

The experiment was conducted in the Municipality of Santiago de Cuba, at Campo Antena Protected Cultivation Facility, on coordinates X: 607547.321; Y: 156420.837, 45.5m above sea level, from America Libre Socialist Company, between November and February. The facility is located near the National Highway, Santiago de Cuba, Km 3 ½, Santa María.

The experimental soil characteristics with pH= neutral to slightly basic, included low contents of organic matter (OM). Phosphorus contents were average; K, Ca²⁺, Mg²⁺ and (Na⁺) showed mid-high values for this type of soil; however, Na⁺ only covered 1% of the exchange complex, producing adverse effects on the crop. All the evaluations were made according to the tables for interpretation of soil analysis (Paneque et al., 2010).

Table 1. Chemical characteristics of the soil (0-20 cm deep)

pH in (H ₂ O)	OM (%)	P (mg Kg ⁻¹)	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺
7.1	2.3	176	(cmol Kg ⁻¹)			
			0.56	0.61	16.3	12.5

pH in H₂O by potentiometer: soil/solution ratio of 1:2.5; OM (organic matter) Walkley-Black, P: solution 0.1 N of H₂SO₄ in the soil/solution ratio of 1: 2.5, Cations NH₄Ac to pH 7 (Paneque et al., 2010).

Experimental design

A completely randomized design was used, with four treatments and four repetitions. A control without inoculation was included in the study; other three were inoculated with strains of *Rhizophagus intraradices* (INCAN-11), *Glomus cubense* (INCAN-4), and *Funneliformis mosseae*. (INCAN-2)

The experimental design was made in four crop cultivation houses, with appropriate conditions for the experiment.

Characteristics of the crop and plot.

1. Crop: Hybrid pepper (v.h.): Locumone.
2. Stage evaluated: Transplantation to final production.
3. Experimental area: Each treatment required a 0.08ha protected cultivation house, for a total experimental area covering 0.32ha.

Plantation management.

Transplantation.

Before transplantation, the area was irrigated to ensure proper soil moisture, and prevent seedling stress. After plantation, the area was irrigated without nutrients to guarantee proper moisture around the roots, and prevent air bubbles between the root ball and the surrounding soil, for quick root development of the seedlings. The seedlings were 32-36 days old, with a mean height of 12 cm, 6 true leaves, and 3 mm stem thickness.

Transplantation was made early in the morning to prevent the occurrence of water stress; the holes in the soil were wider than the root ball, the inoculant was applied before planting. After placing the seedlings in the holes, the soil was slightly pressed around in order to fix the root system.

Density and plantation frame

The plantation frame was 1.20 m x 0.80 m.

Post-transplantation water stress

Following the first irrigation after transplantation, the plantation was submitted to water stress for the first 15 days (AT), with constant monitoring of moisture to favor plant root development, and adequate rooting. Then the fertilizer was irrigated according to the technology established, only inducing stress when the plantation produced more than 75% flowering.

Tutorship

The plantation was induced to produce three stems, with a total of 5 700 shoots, which were tutored technically, at one fruit per axillary bud, to improve overall aeration of the plant, stimulate radiation, and tilling, which effected on the final production, fruit quality, and disease control.

Pruning

Three different pruning types were established after transplantation.

Fruit pruning

First, the fruit from the first bifurcation of the plant was removed, to help plant growth and development. It was done to little exuberant plants, in order to leave just one fruit per branch seeking higher commercial quality fruits. Further pruning was performed to remove deformed, damage, and small, non-commercial fruits.

Formation pruning

The buds formed under the first bifurcation of the plant were removed to make sure that these new buds do not compete over space, water, and nutrients.

Trimming

The nonproductive branches were removed after the plant produced the first fruit, including the branches below the fruit to protect it from possible sunburn or pests, and ensure fruit quality.

Leaf removal

The damaged, sick, or old leaves were removed throughout the plant vegetative cycle.

Decapitation

When the crop cycle was established, 20-30 days before demolition, all the apical buds were decapitated to favor fruit weight and quality.

Harvesting and post-harvesting

The optimum harvesting moment was determined 70 days after transplantation, when the fruit was formed, and was technically ripe (the fruit is dark green). Harvest was performed early in the morning, and late in the afternoon, with shears and knives, to prevent tearing or damaging the fruits and plants. After harvesting, the fruits were handled carefully to ensure commercial quality.

Treatments applied in the protected cultivation houses

- 1 -Control (without inoculation)
- 2 -*Rhizophagus intraradices* (INCAN-11)
- 3 -*Glomus cubense* (INCAN-4)
- 4 -*Funneliformis mosseae* (INCAN-2)

Selection of efficient strains.

Before choosing the strains, native AMF spores were counted. This zigzag sampling was done to all the four cultivation houses engaged in the experiment, and were evaluated according to the Phillip & Hayman (1970) methodology. A number of 652 spores per 100g of soil were determined in the laboratory, which demonstrated the low contents of spores, and therefore, the necessary utilization of efficient AMF strains. This criterion was based on the results of this strain efficiency in other crops.

The mycorrhizal inoculants used in the experiment belong to the AMF collection of the National Institute of Agricultural Sciences (INCA), which are listed below:

- *Glomus intraradices*, now reclassified as *Rhizophagus intraradice*, by Schüßler & Walker (2011)
- *Glomushoi-like*, currently renamed as *Glomus cubense*, according to (Rodríguez et al., 2011)
- *Glomus mosseae*, reclassified as *Funneliformis mosseae* by the previous authors.

These strains were preserved in a substrate developed for this purpose, by the INCA Mycorrhizae Laboratory (Patent registration No. 2264), at 4 °C. The AMF inoculants used in the experiment had an average of 50 spores g⁻¹ of fresh soil, previously certified at INCA Mycorrhizae Laboratory.

Inoculation method at transplantation

Different AMF strains were used, depending on the volume of the root ball, 10% of the root ball of each treatment received the application during transplantation.

When the productive cycle of the crop ended, the roots were collected carefully to protect as many root hairs as possible, to protect the absorbing hairs, and collect as much detailed information as possible.

Root colonization

The root hairs sampled were washed with tap water to remove the soil, and were dried in the air. The thinnest root hairs were separated. Approximately 200 mg of root hairs were dried at 70 °C to determine weight, and were dyed according to the methodology described by Phillips & Hayman (1970). The evaluation was performed using the method of intercepts developed by Giovanetti & Mosse (1980), through which mycorrhizal colonization or colonization frequency was determined. When the sample was taken and analyzed, the data were

calculated according to the formula to transform them into colonization percent.

$$\% \text{ Col} = \frac{\sum (1-5)}{\sum (0-5)} \times 100$$

Visual density

The determination of visual density (VD) was done through the methodology of Trouvelot et al. (1986), which evaluates fungal occupation of each intercept and assigns a level. Then it was calculated according to this formula: $DV = \sum A / \sum Z$.

Where: Z is the sum of the intercept numbers counted in each level, and A is the result of the multiplication of the number of intercepts counted in every level (Z) by the occupation percentage observed.

Spore contents (number of spores per gram of dry soil)

Spore count (u), number of spores per gram of dry soil was done in 50 g samples of soil from the rhizosphere of collected plants, according to the extraction method described by Gerdemann & Nicolson (1963), modified by Herrera, Ferrer, Furrzola & Orozco (1995), which is based on screening and humid decanting of fungal propagulates. The spores were collected on a 40µm mesh. Then they were separated by centrifugation with sucrose and Tween 80, and observed through an optical stereo microscope (20-40x).

Plant height

The height of 20 plants was measured in each replica, totaling 80 per treatment, on days 15, 36, and 57 (AT); the first measurement was done using a ruler, then a measure tape was used.

Stem diameter (mm)

Stem diameter was measured at the base, using a caliper gauge (Mitutoyo 530 – 114 – 200 mm). Similar to height evaluations, stem diameter was evaluated during the final transplantation phase in three moments: at 15, 36, and 57 days after transplantation, in 20 plants per replica, totaling 80 plants per treatment.

Mean height of fruits (cm)

Fruit height was measured with a caliper gauge during the first harvest, 70 days after transplantation.

Average equatorial diameter of the fruits (cm)

Fruit diameter was measured with a caliper gauge during the first harvest, 70 days after transplantation.

Average fresh fruit flesh (g fruit⁻¹)

Fresh fruit flesh was done during the first harvest, 70 after transplantation (AT), using a digital scale (Sartorius BSA1245).

The yield from each treatment was measured according to the average weight of the fruits in grams, and was multiplied by the total number of fruits per plant, and the plant totals in each treatment; the result was converted into tons/hectare.

The economic assessment considered all the direct and indirect costs and expenses made during the experimental stage, in relation to production, supplies (fertilizers for fertirrigation, seeds, organic matter), and treatment with biofertilizers such as *Beauveria bassiana*, arbuscular mycorrhizal fungi) to stimulate crop development and growth, and pepper yields. The economic assessment of the results was done following the methodology of Gregersen & Contreras (1980), which included the following indicators:

-Production cost (PC) in \$/ha

PC=all expenses made (direct and indirect)

-Income (I) in \$/ha

I= PV – PC

Where Production Value (PV)

PV (\$/ha) = yields * sales price

-Cost-effectiveness (CE)

Y= I/ PC

-Cost per produced peso (Vc) in \$

Vc= e /PV

Where: g= total expenses (\$) or production expenses, equivalent to PC.

-Unit cost (Uc) in \$

Uc= TC / PP

-TC= Total cost (PC) en \$/ha

-Yield (Y)

Y= Total production /total area

-PP= Physical production (total yields in t/ha)

The production value (PV) was determined according to the prices of the official wholesale list of Acopio Company in 2016, and the quality of the product.

The following information was used to calculate these indicators:

Sales prices of biofertilizers (\$ kg ⁻¹), based on the Price List of the National Institute of Agricultural Sciences (INCA).	
AMF	\$25.0
<i>Beauveria bassiana</i>	\$20.0
Prices of collected products (\$ thousands), based on the Official Price List of MINAG.	
Pepper seedlings	\$3.040
Tilling and planting rates (\$ ha ⁻¹)	
Ox operator (plowing, harrowing)	\$176.90
Furrowing	\$286.50
Prices of seeds purchased (\$ kg ⁻¹), based on the Official Price List of Seeds of MINAG	
Pepper seeds	\$258.46
Price of organic matter (\$ house ⁻¹), based on the Official Price List.	
Humus (0.32 ha)	\$600.00
Molasses residues (0.32)	\$1000.00
Prices of fertilizers and consumption of water (\$ house ⁻¹), based on the Official Price List)	
Total fertilizer consumption in the period	\$1546.32
Total water consumption in the period	\$514.80
Prices of electricity, telephone in cultivation houses (\$ house ⁻¹)	
Electricity and telephone	\$500.00
Sales price of pepper by category	
First	\$ 6.00 kg
Second	\$ 4.00 kg
Third	\$ 3.00 kg

Statistical analysis

The experimental data of every variable proposed were analyzed through Statgraphics, Centurion. XV.v15.2.14, and analysis of simple variance (ANOVA). Mean comparisons were made according to the multiple range test of Duncan (p ≤ 0.05) The economic assessment of the treatments was performed following the methodology of Gregersen & Contreras (1980). The data collected were processed by simple ANOVA, Duncan multiple mean comparisons test (p≤0.05).

Results and discussion

The Protected Cultivation House Farm at Campo Antena comprises 1.52 ha, and has a total of 23 cultivation houses, with the following specifications: 800 m² (40 m long and 20 m wide). Each house has

10 furrows with a row each; the planting frame is 1.20 m x 0.80 m, and the total number of plants per house is 1900.

AMF performance in the crop

The colonization frequency and visual density of the three strains was observed in fungal performance compared to the controls. A marked trend to higher values of these indicators was observed in *Glomus cubense* (INCAN-4) (54.5 and 3.35), and *Rhizophagus intraradices* (INCAN-11) (41.5 and 1.19), particularly *Glomus cubense* (INCAN-4), as shown in Tables 2 and 3. These results may be determined by the pH in which the process was developed, the most appropriate for these strains. The values may have been influenced by the capacity of AMF efficient strains to set a molecular tie with the microsymbionts, which is closely related to the soil type.

Table 2. Colonization intensity

Colonization intensity (%)	SE _x : 0.1644
Control without inoculation	15.7d
<i>Rhizophagus intraradices</i> (INCAN-11)	41.5 b
<i>Glomuscubense</i> (INCAN-4)	54.5a
<i>Funneliformis mosseae</i> (INCAN-2)	24.4c

Table 3. Visual density index

Visual density index	SE _x : 0.1854
Control without inoculation	0.23d
<i>Rhizophagus intraradices</i> (INCAN-11)	1.19b
<i>Glomus cubense</i> (INCAN-4)	3.34a
<i>Funneliformis mosseae</i> (INCAN-2)	0.37c

Studies done by P.J. Rivera et al. (2015), found the importance of the edaphic environment and pH in relation of AMF strain effectiveness, based on 39 field trials comparing the efficiency of several AMF strains when inoculated into a broad variety of crops and soils. The soils where the trials were performed ranged their pH-H₂O between 4.7 and 7.3, as well as interchangeable Ca contents between 3.5 and 37.2 cmol_c.kg⁻¹ in the first 20 cm of depth, where the pH and interchangeable Ca showed the greatest correlation coefficients to AMF strain effectiveness. Hence, *R. intraradices* (INCAN-11) was the most effective at pH > 7, which decreased as the pH declined, with very low effects under the pH=4.8. On the contrary, *F. mosseae* (INCAN-2) showed the greatest effectiveness with the pH=4.8, and decreased

when the pH increased, with very low effects at the pH > 7. Although *G. cubense* (INCAN-4) showed the greatest effectiveness with the pH between 5.8 and 6.5, it also showed an intermediate performance compared to the rest of the pH values studied.

Moreover, Ruth et al. (2011), when analyzing the indicators of mycorrhizal symbiosis performance in pepper, observed a positive effect after the inoculation of AMF strain on the percentage of root colonization and visual density; the highest values were observed in the *G. cubense* strain. (INCAN-4). It was demonstrated that mycorrhization efficiency is influenced by soil richness; it is inhibited in the presence of high nutrient availability.

Table 4. Spore contents (number of spores per gram of dry soil)

Spore contents (number of spores per gram of dry soil)	
Control without inoculation	45d
<i>Rhizophagus intraradices</i> (INCAN-11)	52b
<i>Glomuscubense</i> (INCAN-4)	58a
<i>Funneliformis mosseae</i> (INCAN-2)	48c

In general terms, an increase in the number of spores was observed in the treatments inoculated, and in relation to the control without inoculation (Table 4), particularly in treatment 3. This result may have been influenced by the pH in the aqueous solution in the soil, since this cultivation system undergoes adjustments in water for irrigation that can modify the pH. Likewise, it can be influenced by the soil atmosphere, and the fertility level, which were analyzed 70 days after transplantation, when the plant develops a greater physiological activity.

Similar results were achieved by Montero et al. (2010) upon evaluation of spores at the beginning (before transplantation) and after harvesting, using two moisture levels. They observed significant differences (p<0.05) among the mycorrhizae treatment, compared to the control without inoculation, in organoponics.

This indicator showed the effects of microorganism inoculation, not only in percentages of colonization, visual density, but also in a greater number of spores in the plant rhizosphere, that ensured greater occupation of the ecological niche.

Rivera et al. (2001) considered that another determining factor of symbiotic activity is the specific type of soil or substrate, or even more, nutrient concentrations or balance in the soil solution, the speed of mineralization of organic matter, the capacity of cationic exchange (CEC), and particularly, the Ca⁺⁺ levels.

The utilization of beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) has become an interesting alternative to enhance growth and development of crops, by reducing the application of fertilizers into the ecosystem. AMF can increase nutrient uptake (N, P, K, Ca, Cu, Mg, Mn, Zn, etc.), since they expand the root exploration area through the extension of hyphae on the soil. However, the beneficial effects of AMF in pepper may vary, in accordance with the AMF strains used (Alonso et al., 2013).

These results are comparable to González et al. (2008) when they demonstrated that treatments inoculated with *G.cubense* (INCAN-4) showed significantly higher mycorrhizal colonization, visual density, and number of spores in the ryzhosphere, than the ones that were not inoculated in brown soils without carbonates. It corroborated the effectiveness of this strain to reach, at least in this experiment, greater root coverage levels than the native AMFs.

Plant growth

Table 5. Plant height

Plant height (m)			
Measurements	First measurement (15 AT)	Second measurement (36 AT)	Third measurement (57 AT)
Control without inoculation	0.32d	0.59d	1.18d
<i>Rhizofagus intraradices</i> (INCAN-11)	0.41b	0.64b	1.41b
<i>Glomus cubense</i> (INCAN-4)	0.47a	0.78a	1.63a
<i>Funneliformis mosseae</i> (INCAN-2)	0.36c	0.64c	1.19c
Note: Means with unequal scripts have significant differences (p≤ 0.05)			
SE _x	0.4507	0.8734	0.0121

Table 6. Plant thickness

Stem thickness (mm)			
Measurements	First measurement (15 AT)	Second measurement (36 AT)	Third measurement (57AT)
Control without inoculation	4.9	9.3	13.9
<i>Rhizofagus intraradices</i> (INCAN-11)	9.4	14.2	19.1
<i>Glomus cubense</i> (INCAN-4)	10.5	18.1	22.6
<i>Funneliformis mosseae</i> (INCAN-2)	7.9	12.4	16.4

SE _x	0.1368	0.1948	0.225
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Tables 5 and 6 show the effect of arbuscular mycorrhizal fungi on the growth variables, which was higher in the mycorrhizal treatments than in the control without inoculation. The greatest plant growth was observed in the treatments inoculated with *R. intraradices* (INCAN-11) and *G. cubense* (INCAN-4), the latter with the best results, with average heights of 0.47, 0.79, and 1.61 m, and average stem thickness of 12, 19, and 22.7 mm, at 15, 36, and 57 days after transplantation (AT), respectively.

Similar results were achieved by Angulo et al. (2018) in some global growth parameters evaluated in Bell Pepper, and Jalapeno Pepper, respectively. The AMF inoculated treatments were observed to induce greater plant height in the two Chile cultivars. In Bell Pepper, the H1 treatment was higher (p≤ 0.05) than the other treatments, whereas, in Jalapeno, treatments H1 and H3 induced greater plant height.

Angulo et al. (2018) commented that AMFs are important in ecological agriculture thanks to the benefits of establishing a symbiosis with the plant, acting as mobilizers of water and nutrients, such as phosphorus, zinc, and copper, and as agents of biological control.

These results are comparable to the ones described by R. Rivera et al., (2015) who referred that stem diameter and height, a parameter that can offer information about plant vigor, showed statistically significant differences among the strains studied. The greatest effect was observed in *G. cubense* (INCAN-4), in relation to *G. mosseae* (INCAN-2) and the control. Generally, their research show differentiated stem growth at sampling; the plants responded to inoculation with different AMF species. A differentiated effect was observed among strains, with the greatest increases in stem height and diameter when *G. cubense* was inoculated. Another aspect that influences crop growth and development is the presence of compound exudates through AMFs, that stimulate microbial activity in the ecological niche.

Likewise, Alonso et al., (2013) highlighted that the effect observed in AMFs on stem diameter in plants of *Capsicum annum* L. may be caused by greater efficiency in nutrient absorption, especially P, by inoculated plants, in comparison to the controls. Mycorrhizal plants grow better than nonmycorrhizal plants in infertile soils, since mineral nutrition increases through the action of hyphae, which assist in exploring a higher volume of soil than root hairs of plants, facilitating growth.

The results achieved by Charles & Martín (2015) in tomato, describe the behavior of plant height at 30 and 45 days after transplantation. Assessment of plant height at 30 days after transplantation showed that treatments 9 (50% mineral fertilizer + humus +AMF), 13 (100% mineral fertilizer + humus +AMF), 14 (100% mineral fertilizer + without humus + AMF), and 15 (100% mineral fertilizer + humus + without AMF), had no significant differences among them, due to the effect of AMF on nutrient availability.

Growth variables

Table 7. Fruit height (cm)

Average fruit height (cm)	
Control without inoculation	6.6d
<i>Rhizofagu sintraradices</i> (INCAN-11)	10.2b
<i>Glomus cubense</i> (INCAN-4)	14.2a
<i>Funneliformis mosseae</i> (INCAN-2)	7.2c

Table 8. Equatorial diameter of the fruit (cm)

Equatorial diameter of the fruit (cm)	
Control without inoculation	5.4d
<i>Rhizofagus intraradices</i> (INCAN-11)	8.1b
<i>Glomus cubense</i> (INCAN-4)	10.1a
<i>Funneliformis mosseae</i> (INCAN-2)	5.9c

The AMF treatment showed an adequate behavior compared to the control without inoculation (Tables 7 and 8), particularly, T3, with the best values, which evidences that the interaction of symbionts enhance pepper development.

A physiological analysis of the productive performance of any crop species shows that increases in the growth variables and yields are closely related to photosynthetic process occurring in the leaves, so the analysis of morphological variables linked to this process is critical. R. Rivera et al., (2015) pointed out the advantages of AMF efficient strain inoculation, since it stimulates growth, contributing with nutritional benefits, and protection against soil pathogens.

Table 9. Fruit weight (g)

Fruit weight (g)	
Control without inoculation	153.7d
<i>Rhizofagus intraradices</i> (INCAN-11)	261.58b
<i>Glomus cubense</i> (INCAN-4)	317.6a
<i>Funneliformis mosseae</i> (INCAN-2)	172.7c

Treatments 2 and 3 showed the best performance, with values of 261.58 and 317.6 grams, respectively; T3 had the best results, as shown in Table 9. Treatments 1 and 4 showed the lowest values, which might be directly linked, in treatment 1 (control), to nutrient availability and consumption in the saturated aqueous extract in the soil used during the process. It influenced the accumulation of salts or nutrient

deficit, affecting plant development and yields. In treatment 4 (*F. mosseae*), the result could have been determined by the edaphic environment, which did not allow for competition with the local biota.

Waterer and Coltman (1988) said that inoculation with arbuscular mycorrhizal fungi promote flowering and fruiting of pepper plants, by improving P intake. Besides, AMFs can influence in flowering, altering the hormonal balance of the host, and increase production of total dry matter, and fruit yields.

Arbuscular mycorrhizal fungi are a promising microbiological supply for sustainable agriculture, their role in the function of ecosystems and their potential as biological fertilizers are sufficient reasons to consider them important components of modern agroecology (Rivera et al., 2007 and Gamboa 2015).

Table 10. Yields (t/ha)

Crop yields in tons per hectare (t/ha)	
Control without inoculation	43.75
<i>Rhizofagus intraradices</i> (INCAN-11)	73.75
<i>Glomus cubense</i> (INCAN-4)	90
<i>Funneliformis mosseae</i> (INCAN-2)	48.75

The effect of a greater number of spores undergone by the pepper plants treated with AMF caused the plants in this treatment to have better water condition as part of the benefit brought by symbiosis, than the control plants, which was even more significant at the end of the crop cycle.

Regarding yields, (Table 10), treatments 3 and 2 showed the best performance, the latter went over the national mean, which demonstrates that in the conditions of the experiment, the best results were achieved with the inoculation of AMF, compared to the control.

Netto (2008) pointed that the highest benefit observed from AMF use is its role in plant nutrition, caused by a marked increase in nutrient absorption and translocation processes, mass flow of diffusion, the effects on growth, and the production of plants.

Table 11. Economic assessment of results

T	CT	VP	G	R	Cv	Cu
1	6.262	9.100	2.837	0.45	0.31	1.789
2	6.312	19.072	12.759	2.02	0.66	1.059
3	6.309	26.064	19.754	3.13	0.75	0.871
4	6.309	11.397	5.087	0.80	0.44	1.605

T: Treatments

T.C: Total cost

P.V: Production value

P: Profit

CE: Cost-effectiveness

Vc: Variable cost

Uc: Unit cost

Treatment 3 (*G. cubense*) (INCAN-4) showed the best economic indicators, with a 3.13 profitability, which means that out of every peso spent, the company profited \$3.13. The second treatment (*R. intraradices*) (INCAN-11) had a positive performance, with a profit margin of \$12 759. These results show that the utilization of biofertilizers and adequate amounts of fertirrigation in crop farming production are efficient, both in terms of proper ecosystem management and profits. The economic indicators of the four treatments were favorable, however, despite the low yields in treatments 1 and 4, no financial losses were observed.

AMFs have proven their efficiency in crop farming systems; studies have evidenced satisfactory results in several different crops with economic interest, and types of soils. They contribute to better utilization of soil nutrients by plants, apart from protecting against pathogens, increasing water stress tolerance, and increasing crop yields between 30 and 45% (Rivera et al., 2007).

Conclusions

The AMF strains applied had a favorable behavior in relation to the indicators evaluated: growth, development, and crop yield.

The application of *G. cubense* produced the best pepper (*Capsicum annum L.*) yields.

Author contribution

1. Beatriz Toledo Cabrera: experimental design, data analysis, interpretation, and collection, redaction of the manuscript, final review.
2. Gerardo Montero Limonta: research planning, analysis and interpretation of results, review of the manuscript.
3. Anieska Bazán Delgado: analysis, interpretation and redaction of the manuscript, creation of the template, final review.

Conflicts of interest

Not declared.

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